Figure S1. Related to Figure 1

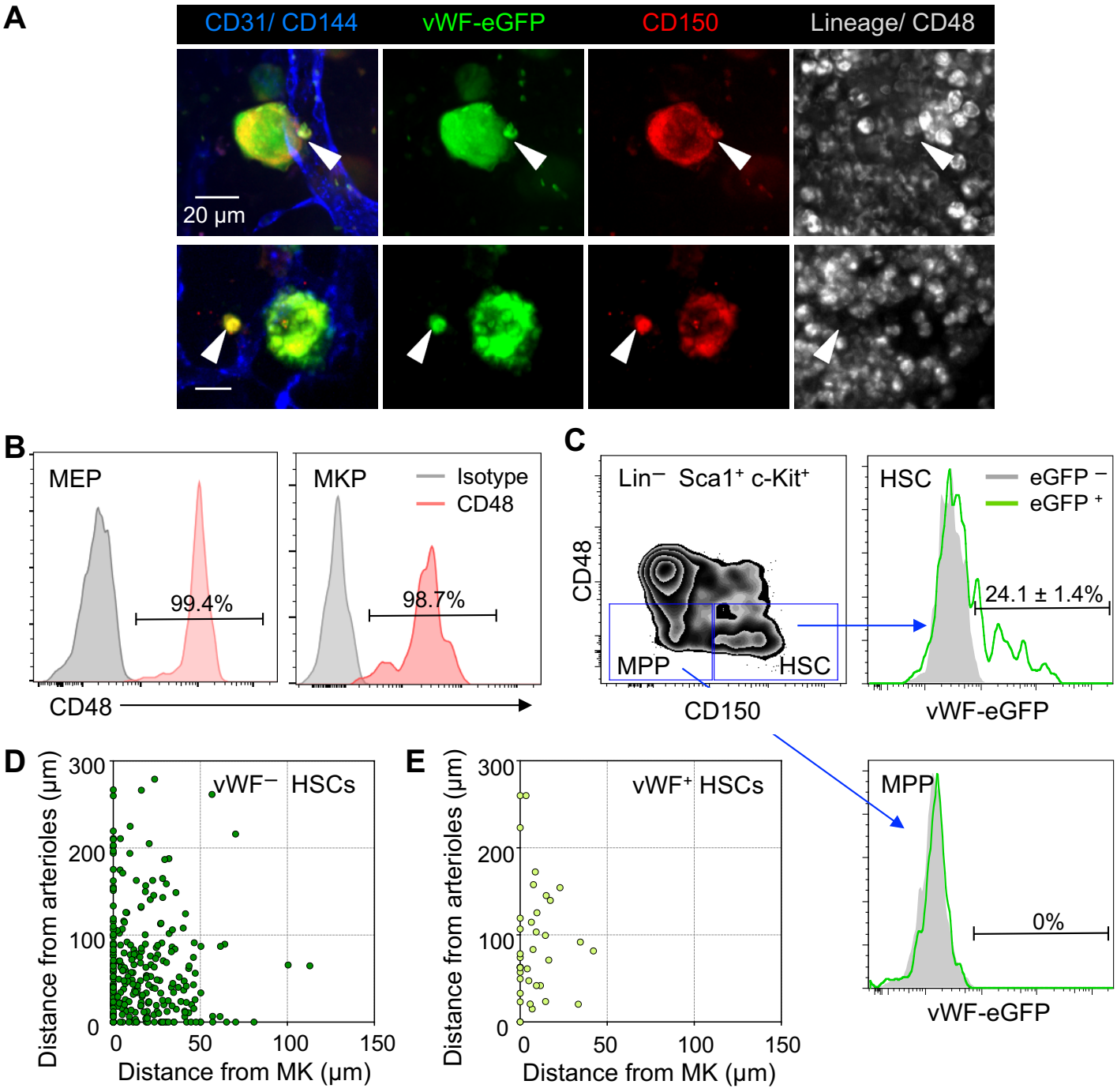

Figure S1, related to Figure 1. vWF-eGFP⁺ cells constitute a small fraction of the LSK CD48⁻ CD150⁺ HSC population in the mouse BM

(**A**) Representative whole-mount images of vWF-eGFP+ HSCs in the mouse sternal BM. White arrowheads denote phenotypic Lineage (Lin)⁻ CD48⁻ CD150⁺ vWF-eGFP⁺ HSCs. MK are distinguished by their size, morphology and CD150 and vWF expression. Vascular endothelial cells are stained intravenously with antibodies to CD31 and CD144. (**B**) Bone marrow FACS analysis of CD48 expression in MK/erythroid progenitors (MEP; Lin⁻ Sca1⁻ c-Kit⁺ FcRII/III⁻ CD34⁻) and MK progenitors (MKP; Lin⁻ Sca1⁻ c-Kit⁺ CD150⁺ CD41⁺) (**C**) FACS gating strategy for the isolation of phenotypic Lin⁻ Sca1⁺ c-Kit⁺ CD48⁻ CD150⁺ vWF-eGFP⁺ HSCs from the BM of *Vwf-eGFP* mice. (**D**) 2D distribution of the distances between vWF-eGFP⁻ (**D**) and vWF-eGFP⁺ HSCs (**E**) and MK or arterioles in the mouse sternal BM. *n* = 282 vWF⁻ HSCs and *n* = 37 vWF⁺ HSCs (related to **Figure 1C** and **1D)**.

Figure S2. Related to Figure 2

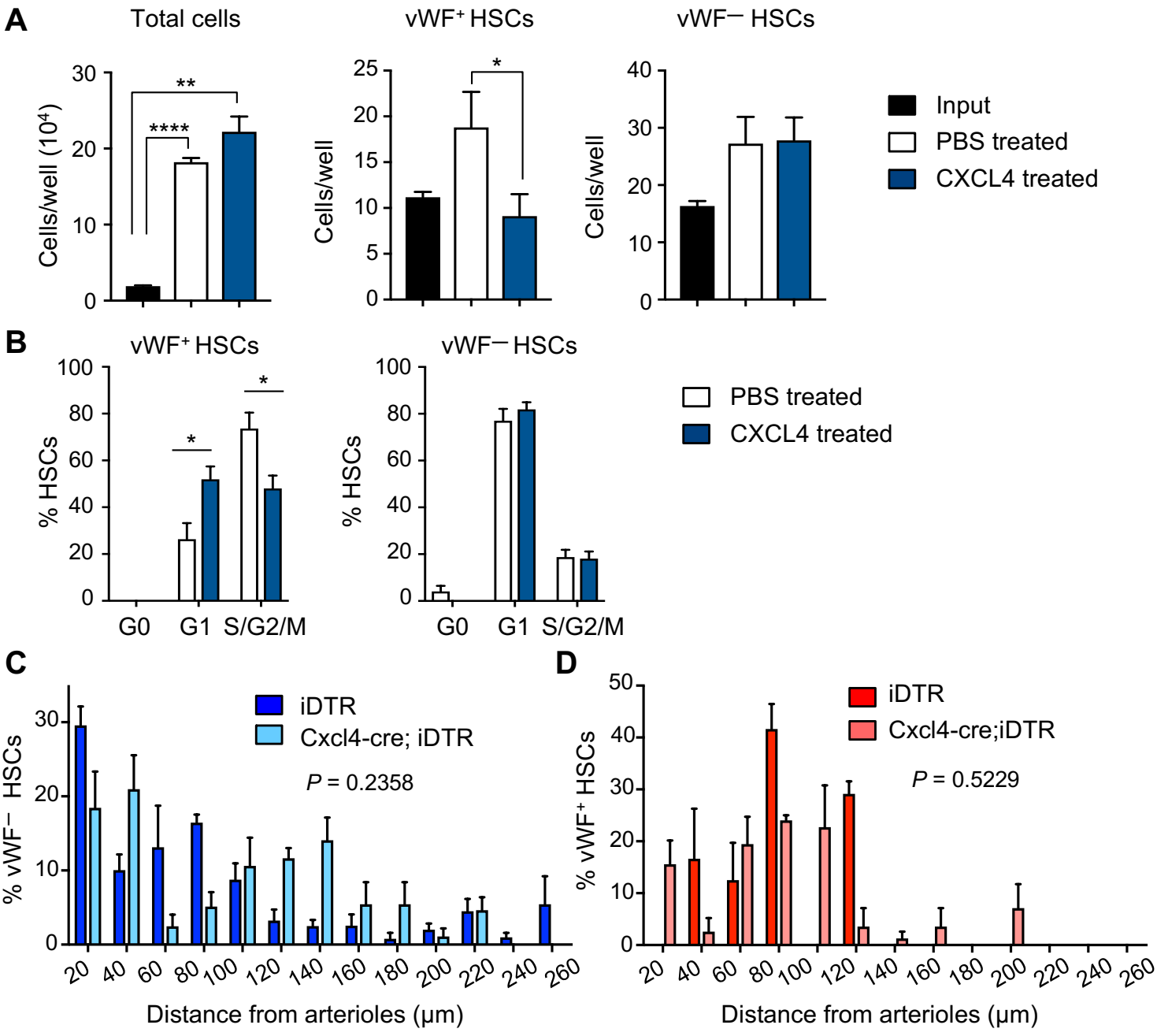

Figure S2, related to Figure 2. Distribution of lineage-biased HSCs in the mouse BM after MK depletion

(**A**) Absolute number of cells per well in cultures of Lineage (Lin)$^-$ cells isolated from *Vwf-eGFP* mice and cultured *in vitro* during 4 days in the presence or absence of recombinant CXCL4. Input indicates the number of cells on day 0. *n* = 10 wells per group (**B**) Cell cycle analysis by FACS using anti-Ki-67 and Hoechst 33342 staining of vWF$^-$ and vWF$^+$ HSCs *in vitro* cultured in the presence or absence of CXCL4. *n* = 8 (PBS) and *n* = 5 (CXCL4) wells per group. *P < 0.05, **P < 0.01, ****P < 0.0001. Error bars S.E.M.. Unpaired Student's *t* tests. Localization of phenotypic Lin$^-$ CD48$^-$ CD150$^+$ vWF-eGFP$^-$ (**C**) and vWF-eGFP$^+$ (**D**) HSCs in the mouse BM relative to arterioles in control (*iDTR;Vwf-eGFP*) and *Cxcl4-cre;iDTR;Vwf-eGFP* mice, 7 days after DT treatment. *n* = 142 vWF$^-$ and *n* = 14 vWF$^+$ HSCs from control *iDTR;Vwf-eGFP; n* = 83 vWF$^-$ and *n* = 35 vWF$^+$ HSCs from *Cxcl4-cre;iDTR;Vwf-eGFP. P* values were determined by two-sample KS test. Error bars S.E.M.

Figure S3. Related to Figure 2

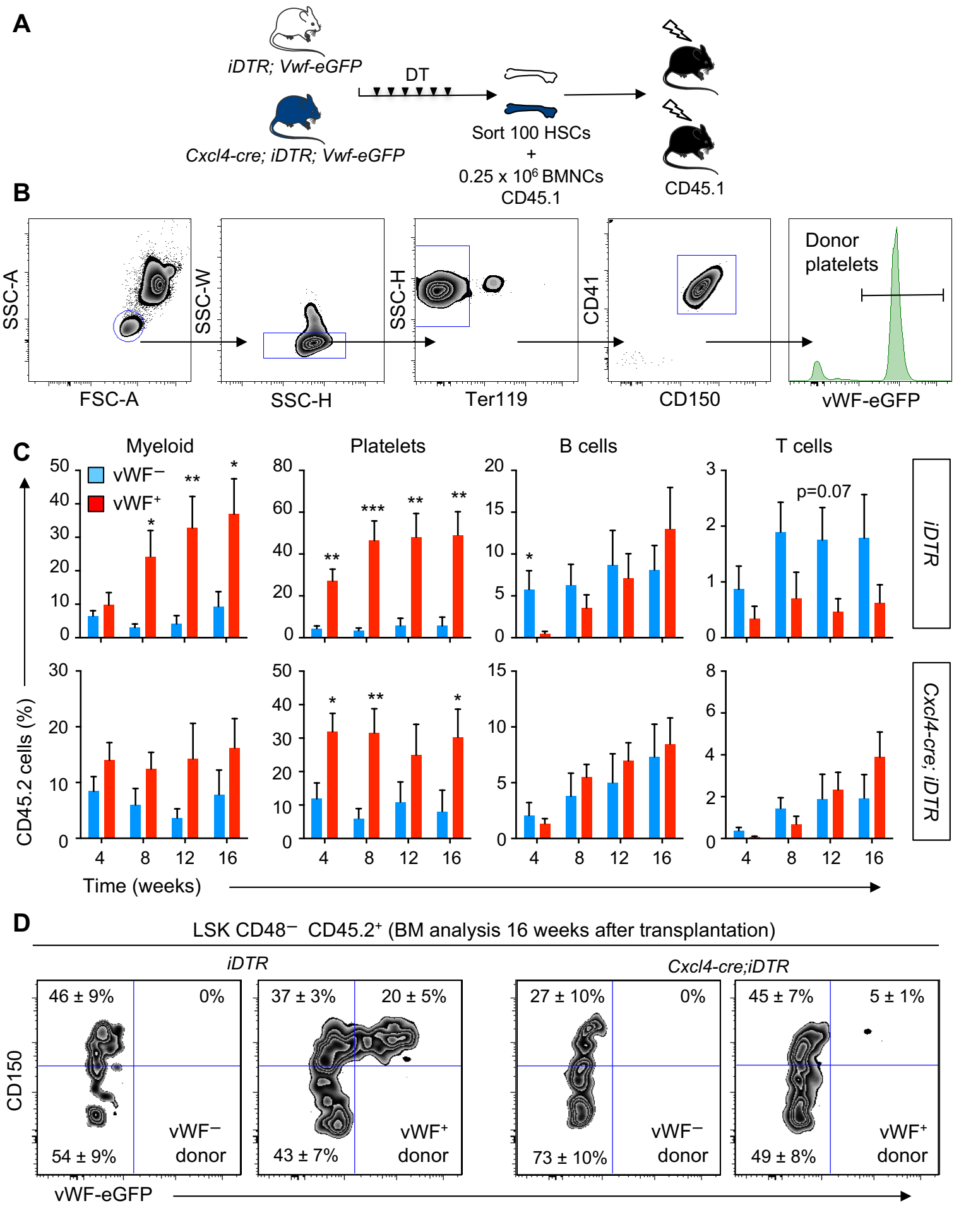

Figure S3, related to Figure 2. Reconstitution potential of lineage-biased HSCs after MK depletion

(**A**) Experimental design of the competitive reconstitution assays used to determine the effect of MK depletion in the lineage potential of purified vWF^{-} and vWF^{+} HSCs. (**B**) Gating strategy for peripheral blood platelet reconstitution analysis in mice 16 weeks after competitive transplantation of CD45.2 vWF^{+} HSCs. (**C**) Quantification of tri-lineage (myeloid, B cell and T cell) and platelet engraftment in the peripheral blood of mice analyzed in **Figure 2H-J.** *n* = 8 (iDTR vWF^{-} and vWF^{+} groups); *n* = 9 (Cxcl4-cre,iDTR vWF^{-} group); *n* = 8 (Cxcl4-cre,iDTR vWF^{+} group). (**D**) Representative BM FACS plots showing donor HSCs contribution to recipient CD45.2^{+} LSK CD48^{-} CD150^{+} vWF-eGFP^{-} or vWF-eGFP^{+} HSC compartment, at 16 weeks from the mice analyzed in **Figure 2H-J.** *P < 0.05, **P < 0.01. Error bars S.E.M. Unpaired Student's *t* tests (C).

Figure S4. Related to Figure 3

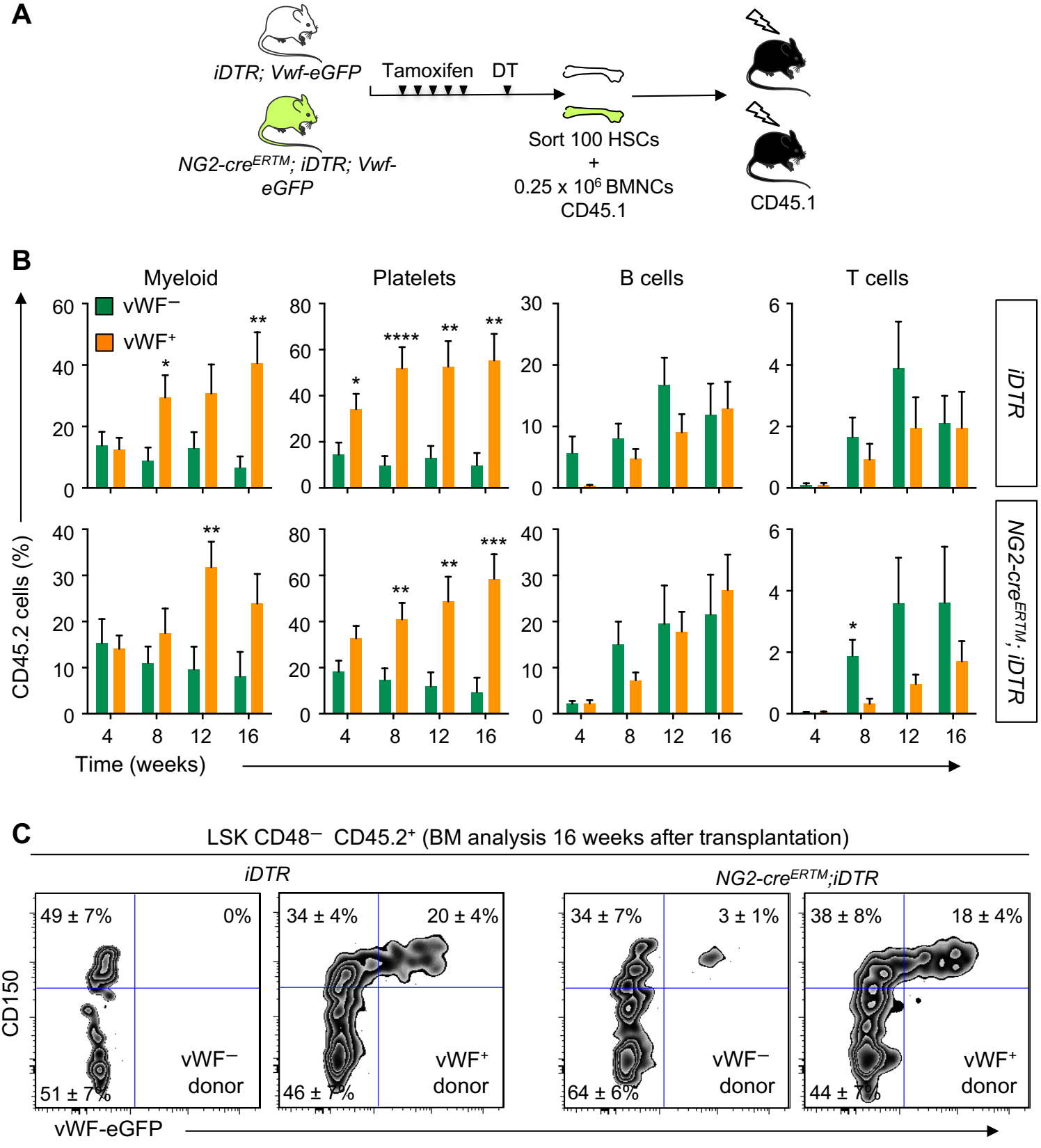

Figure S4, related to Figure 3. Reconstitution potential of lineage-biased HSCs after NG2+ cells depletion

(**A**) Experimental design of the competitive reconstitution assays used to determine the effect of $NG2^{+}$ cells depletion in the lineage potential of purified vWF^{-} and vWF^{+} HSCs. (**B**) Quantification of tri-lineage (myeloid, B cell and T cell) and platelet engraftment in the peripheral blood of mice analyzed in **Figure 3F-H.** *n* = 8 (*iDTR* groups) and *n* = 9 (*NG2-cre^{ERTM};iDTR* groups. (**C**) Representative BM FACS plots showing donor HSCs contribution to recipient $CD45.2^{+}$ LSK $CD48^{-}$ $CD150^{+}$ vWF-eGFP^{-} or vWF-$eGFP^{+}$ HSC compartment at 16 weeks from the mice analyzed in **Figure 3F-H.** *P < 0.05, ***P < 0.001. Error bars S.E.M. Unpaired Student's *t* tests (B).